Antibody Purification from Western Blotting

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[Abstract] This protocol describes a method of purifying antibodies from sera with denatured antigens immobilized on western blot membranes. Advantages include (1) fast and easy; (2) purification of antibody with antigen in denatured form allows high yield in case antigen protein solubility is limited. Disadvantage is that possible antibodies that recognize certain 3D structure in solution of antigens might not be purified using such a method. Regarding this issue, the flow through is recommended to be kept and can be used for other purification methods with folded antigens.

Materials and Reagents

1. Antigen protein
2. PVDF or Nitrocellulose membrane (Amersham biosciences/GE Healthcare Dharmacon)
3. Ponceau S (Sigma-Aldrich, catalog number: P7170)
4. non-fat Milk powder (Carnation, any your favorite brand in local store)
5. Tween 20 (Sigma-Aldrich)
6. Glycine
7. Acetic acid
8. 2 M Tris-HCl (pH 8.5)
9. NaCl
10. KCl
11. Na_2HPO_4
12. KH2PO4
13. Ultra pure water
14. TBST (see Recipes)
15. 10x TBS (1 L) (see Recipes)
16. PBS (pH 7.4) (see Recipes)
17. Ponceau S solution (see Recipes)

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Equipment

1. Dialysis bag (Thermo Fisher Scientific/Pierce Antibodies, catalog number: 68035)
2. Comb

Procedure

1. Load 1-10 mg purified antigen protein into appropriate SDS-PAGE gel.
   Note 1: High amount of antigen protein is essential to retrieve antibody in high purity and yield. To load as much protein into the gel as possible:
   a. *Use wide comb (vendors normally have comb with only two wells: one well is in regular size for marker, the other well takes the rest of the space).*
   b. *Insoluble protein can be purified under denaturing conditions.*
   c. *Even for soluble protein, higher yield can be achieved by eluting directly from the purification beads by SDS loading buffer.*
   Note 2: If your protein has a big tag such as GST, MBP, NusA, you should always run a preclearance of antibody against these tags from the serum with blot containing these tag proteins.
2. Transfer to PVDF or Nitrocellulose membrane (*note, if your protein size is small, choose 0.2 μm pore size rather than 0.45 μm*).
3. Stain the membrane with Ponceau S solution and cut the strip containing your antigen protein as small as possible.
4. Pre-elute the membrane with 100 mM glycine (pH 2.5) for 10 min. Rinse with TBST.
5. Block with 5% non-fat milk in TBST for 1 h at room temperature (RT).
6. Rinse with TBST three times.
7. Incubate the 5-10 ml antiserum overnight at 4 °C. Depending on the membrane size.
8. Wash with TBST three times.
9. Elute the antibody by incubating the membrane with 1-2 ml 100 mM Glycine (pH 2.5) for 2 min.
10. While incubating, add 75 μl-100 μl 2 M Tris-HCl (pH 8.5) to tubes. The volume should allow a final concentration of 150 μM Tris after step 11.
11. After 2 min, add 1-2 ml of the eluted antibody in glycine from step 9 to the tubes from step 10. The Tris (pH 8.5) should neutralize the acidic glycine.
12. Repeat step 9-11 for three times.
13. Dialyze the fractions against PBS (pH 7.4) and determine antibody concentration as your regular protein work.
14. Test the antibody with western, immunoprecipitation and immunofluorescence.

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Recipes

1. 10x TBS (1 L)
   24.23 g Tris
   80.06 g NaCl
   Mix in 800 ml water
   pH to 7.6 with HCl
   Adjust volume to 1 L
2. TBST (1 L)
   100 ml of 10x TBS
   900 ml ultra pure water
   500 µl Tween 20
   Note: Tween 20 is very viscous and will stick to the tip of your measuring pipettes. Be sure you take the right amount and add in buffer by pipetting up and down. At the same time, the buffer should be stirring.
3. PBS (pH 7.4) (1 L)
   8 g of NaCl
   0.2 g of KCl
   1.44 g of Na_2HPO_4
   0.24 g of KH_2PO_4
   800 ml H_2O
   Adjust pH to 7.4
   Adjust volume to 1 L with additional distilled H_2O
   Sterilize by autoclaving
4. Ponceau S solution (1 L)
   1 g Ponceau S
   50 ml Acetic acid
   Add H_2O to 1 L

Acknowledgments

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References

1. Kurien, B. T. (2009). Affinity purification of autoantibodies from an antigen strip excised from a nitrocellulose protein blot. *Methods Mol Biol* 536: 201-211.

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